

C L A I M S

5 1. A method for identifying the presence of a BBB-specific protein or fragment thereof in endothelial cells of brain capillaries, characterized in that

10 a) endothelial of brain capillaries freshly isolated from brain are conventionally pre-purified by enzymatic digestion,

15 b) the digest obtained in step a) is treated with a lysis buffer that essentially destroys erythrocytes and apoptotic cells present and maintains at least 70% of the endothelial cells of brain capillaries in vital form,

20 c) the product obtained in step b) is optionally purified further,

d) a subtractive cDNA library is prepared from the endothelial cells of brain capillaries and a subtractive tissue,

25 e) a cDNA subtraction is performed using one or more differential hybridization(s),

30 f) clones from the subtractive cDNA library are verified by differential hybridization with respect to their respective expression,

g) the cDNA sequence is completed for the BBB-specific clones from the subtractive cDNA library and

h) the expression pattern of the investigated clones is compared between fresh and cultured endothelial cells of brain capillaries and, that way, the presence of BBB-specific proteins or fragments thereof is identified.

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2. The method according to claim 1, characterized in that the lysis buffer in step b) has the following composition:

Na ⁺	30.0 mM to	60.0 mM
K ⁺	5.0 mM to	7.5 mM
NH ₄ ⁺	80.0 mM to	100.0 mM
Ca ²⁺	1.0 mM to	2.0 mM
Mg ²⁺	6.0 mM to	9.0 mM
Cl ⁻	125.0 mM to	175.0 mM
HCO ₃ ⁻	4.5 mM to	6.5 mM
H ₂ PO ₄ ⁻	0.5 mM to	2.5 mM
SO ₄ ²⁻	0.3 mM to	0.6 mM
HPO ₄ ²⁻	0.4 mM to	0.7 mM
Glucose	1.5 mM to	3.0 mM

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3. The method according to claim 2, characterized in that the lysis buffer has the following composition:

NaCl	30 mM	to	50 mM
KCl	4.5 mM	to	5.5 mM
NH ₄ Cl	80 mM	to	100 mM

CaCl ₂	1.0 mM	to	2.0 mM
MgCl ₂	0.6 mM	to	0.8 mM
MgSO ₄	0.3 mM	to	0.6 mM
NaHCO ₃	4.5 mM	to	6.5 mM
NaH ₂ PO ₄	0.2 mM	to	0.45 mM
Na ₂ HPO ₄	0.4 mM	to	0.65 mM
KH ₂ PO ₄	0.1 mM	to	0.15 mM
Glucose	1.5 mM	to	3.0 mM

4. The method according to one of claims 1 to 3, characterized in that the subtractive tissue in step f) are aortic endothelial cells.

5. The method according to one of claims 1 to 4, characterized in that the complete cDNA sequence in step i) is prepared by screening cDNA libraries and RACE-PCR.

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6. The method according to one of claims 1 to 5, characterized in that the endothelial cells of brain capillaries are derived from man or pig.

15 7. A protein with BBB-specificity or a fragment thereof, obtainable according to a method according to one of the claims 1 to 6.

20 8. The protein according to claim 7, characterized in that it has a sequence selected from SEQ ID NO: 5, SEQ ID NO: 14, SEQ ID NO: 19, or SEQ ID NO: 53.

9. A method for the identification of a presence of a BBB-specific protein or fragment thereof in endothelial cells of brain capillaries, characterized in that

5 a) endothelial cells of brain capillaries freshly isolated from brain are conventionally pre-purified by enzymatic digestion,

10 b) the digest obtained in step a) is treated with a lysis buffer that essentially destroys erythrocytes and apoptotic cells present and maintains at least 70% of the endothelial cells of brain capillaries in vital form,

15 c) the product obtained in step b) is optionally purified further,

20 d) the product obtained in step c) is solubilized in a suitable buffer,

25 e) an isoelectric focusing is performed,

f) the samples from the isoelectric focusing are separated in the second dimension according to the molecular weight,

25 g) differential spots are identified and isolated,

h) mass spectrometric analysis is performed with the isolate of g), and

30 i) an evaluation thereof is constructed via specific database analysis.

10. The method according to claim 9, characterized in that a
35 lysis buffer in step b) has the following composition:

Na ⁺	30.0 mM	to	60.0 mM
K ⁺	5.0 mM	to	7.5 mM
NH ₄ ⁺	80.0 mM	to	100.0 mM
Ca ²⁺	1.0 mM	to	2.0 mM
Mg ²⁺	6.0 mM	to	9.0 mM
Cl ⁻	125.0 mM	to	175.0 mM
HCO ₃ ⁻	4.5 mM	to	6.5 mM
H ₂ PO ₄ ⁻	0.5 mM	to	2.5 mM
SO ₄ ²⁻	0.3 mM	to	0.6 mM
HPO ₄ ²⁻	0.4 mM	to	0.7 mM
Glucose	1.5 mM	to	3.0 mM

11. The method according to claim 10, characterized in that
5 the lysis buffer has the following composition:

NaCl	30 mM	to	50 mM
KCl	4.5 mM	to	5.5 mM
NH ₄ Cl	80 mM	to	100 mM
CaCl ₂	1.0 mM	to	2.0 mM
MgCl ₂	0.6 mM	to	0.8 mM
MgSO ₄	0.3 mM	to	0.6 mM
NaHCO ₃	4.5 mM	to	6.5 mM
NaH ₂ PO ₄	0.2 mM	to	0.45 mM

Na ₂ HPO ₄	0.4 mM	to	0.65 mM
KH ₂ PO ₄	0.1 mM	to	0.15 mM
Glucose	1.5 mM	to	3.0 mM

12. A protein with BBB-specificity or a fragment thereof, obtainable according to a method according to one of claims 9 to 11.

13. The protein according to claim 12, characterized in that it has a sequence selected from SEQ ID NO: 23, SEQ ID NO: 27, SEQ ID NO: 33.

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14. A use of a protein according to one of the claims 7 to 8 or 12 to 13 for the preparation of a medicament for the transport of substances through the blood-brain barrier.

15 15. The use of a protein according to one of claims 7 to 8 or 12 to 13 for the preparation of an agent or medicament for the diagnosis or therapy of diseases that are based on a dysfunction of the blood-brain barrier.

20 16. An agent for the diagnosis of diseases that are based on the dysfunction of the blood-brain barrier, characterized in that it comprises a protein according to one of the claims 7 to 8 or 12 to 13.

25 17. The agent for the therapy of diseases which are based on a dysfunction of the blood-brain barrier, characterized in that it comprises a protein according one of claims 7 to 8 or 12 to 13.

30 18. A use of one or more DNA-sequence(s) selected from SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 15, SEQ ID NO: 22, SEQ ID

NO: 23, SEQ ID NO: 26, SEQ ID NO: 32, SEQ ID NO: 35, SEQ ID
NO: 36, SEQ ID NO: 43, SEQ ID NO: 49, SEQ ID NO: 52, SEQ ID
NO: 54, SEQ ID NO: 55, for the preparation of an agent for
the diagnosis of diseases which are connected with ischemic
5 conditions.

19. The use according to claim 18 for the diagnosis of stroke, myocardial infarction or tumor-associated conditions.
- 10 20. The use according to one of claims 18 or 19, characterized in that the diagnosis is carried out via the control of the expression of the proteins encoded by said DNA-sequences.